

# Indisulam efficacy studies in in vivo models

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Targeting the spliceosome through RBM39 degradation results in exceptional responses in high-risk neuroblastoma models

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## Detailed protocol

### Indisulam-formulation

This protocol has been developed for indisulam-formulation and mice treatment with intraperitoneal and intravenous injection.

1. A person must be aware of the relevant health and safety issues before preparing and administering indisulam. The indisulam-formulation and objects used in preparation may be hazardous to the person preparing it or to people who encounter it, therefore the necessary precautions must be taken in drug preparation and disposal to minimize the risk.
2. Indisulam, (molecular weight: 385.86) was purchased from MedKoo Biosciences (catalog # 201540). Stock solution (300 mM) was made in DMSO (Fisher, BP231-100-100 ml) by vortexing and sonication, up until it is mixed properly and appears as clear dark red solution. Use water bath sonicator at 4 C.
3. Formulation: Vehicle or indisulam for animal injection was made using recipe, 3.5% DMSO/indisulam-stock, 6.5% Tween 80 (Sigma, P8192-10mL), 90% saline (Sigma, S8776-100mL). It is important to add reagents sequentially. First, add DMSO/indisulam-stock, then Tween-80, vortex the mixture and then add saline with vortexing for 5-10 seconds.
4. Before formulation preparation, it is essential to calculate the dose/volume correctly.
5. Making the indisulam-formulation for 4 mice (add these in one eppendorf tube): Indisulam 17.2 µl (from stock 300 mM), DMSO 10.8 µl, Tween-80 52 µl, vortex the mixture then Saline: 720 µl, pipette up and down, vortex the mixture for 5-10 seconds. Alternatively, sonicate the drug for 10 minutes until the color appears clear pink. Each mouse injected intravenously with 200 µl/20g mouse body weight of formulation (dose 25 mg/kg).
6. Aliquot the indisulam-formulation into 1.5 mL Eppendorf tubes and store them at -20 C. (It is good for 2-3 weeks at -20 C).
7. On the day of indisulam administration, either take fresh preparation or stock. Keep away from light.
8. All murine experiments were done following a protocol approved by the Institutional Animal Care and Use Committee of St. Jude Children's Research Hospital. Subcutaneous xenografts were established in CB17 SCID mice (CB17 scid, Taconic) or NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG; T1 Jackson Laboratory) mice.

### Materials and Reagents

DMSO

Indisulam

Tween 80

### Equipment

Water bath sonicator

BD syringes

-20 C refrigerator

### References:

1. Singh, Shivendra, et al. "Targeting the spliceosome through RBM39 degradation results in exceptional responses in high-risk neuroblastoma models." *Science advances* 7.47 (2021): eabj5405.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Singh, S. , Quarni, W. , Nijhawan, D. , Davidoff, A. and Yang, J. (2022). Indisulam efficacy studies in in vivo models. Bio-protocol Preprint. [bio-protocol.org/preprint1862](https://bio-protocol.org/preprint1862).
2. Singh, S., Quarni, W., Goralski, M., Wan, S., Jin, H., Velde, L. V. D., Fang, J., Wu, Q., Abu-Zaid, A., Wang, T., Singh, R., Craft, D., Fan, Y., Confer, T., Johnson, M., Akers, W. J., Wang, R., Murray, P. J., Thomas, P. G., Nijhawan, D., Davidoff, A. M. and Yang, J. (2021). Targeting the spliceosome through RBM39 degradation results in exceptional responses in high-risk neuroblastoma models. *Science Advances* 7(47). DOI:

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